A Protective Role for the Fifth Complement Component (C5) in Allergic Airway Disease

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Rationale: Reports from our laboratory, as well as those from others, have documented the importance of complement activation, the C3a anaphylatoxin, and its receptor, C3aR, in promoting Th2 effector functions in a mouse model of bronchopulmonary allergy. Although deficiency in the fifth complement component (C5) has been linked to enhanced airway hyperresponsiveness in mice, the contribution of C5 to other major biological hallmarks of asthma has not been evaluated.
Objective: Accordingly, congenic C5-sufficient and C5-deficient mice were subjected to a mouse model of bronchopulmonary allergy to assess the impact of C5 on pulmonary inflammation and Th2 effector functions in experimental asthma.
Methods and Main Results: In contrast to observations reported for C3- and C3aR-deficient animals, C5-deficient mice exhibited significantly increased airway hyperresponsiveness relative to wild-type congenic control mice after antigen challenge. Moreover, challenged C5-deficient mice had a 3.4-fold and 2.7-fold increase in the levels of airway eosinophils and lung interleukin (IL)-4-producing cells, respectively, compared with challenged wild-type mice. Consistent with the numbers of IL-4-producing cells, C5-deficient mice also had increased bronchoalveolar lavage levels of the Th2 cytokines IL-5 and IL-13 and elevated serum levels of total and antigen-specific IgE.
Conclusions: These data indicate that C5 plays an important protective role in allergic lung disease by suppressing inflammatory responses and Th2 effector functions observed in this experimental model. The protection provided by the presence of C5 is likely mediated by C5a, suggesting that C5a may play a significant role in tempering inflammation in Th2-driven diseases such as asthma.

Keywords: allergy; complement; lung; Th1/Th2 cells; T lymphocytes

Asthma is a complex disease of the lung characterized by airflow obstruction, airway hyperresponsiveness (AHR), and airway inflammation. Current research efforts have focused on CD4+ type 2 T-helper cells (Th2) and their pivotal role in the pathogenesis of this disease (1). Through the release of interleukin 4 (IL-4), IL-5, and IL-13, Th2 cells contribute to bronchial hyperreactivity, eosinophil recruitment, and mucus hypersecretion (2–4), as well as initiating B-lymphocyte differentiation and production of IgE antibodies (5, 6).

Recent evidence has also documented a role for the complement system in the pathology of asthma. Complement activation

peptides have been detected in the lungs of patients with asthma (7–10). Studies have also shown that complement activation can contribute to the development of AHR (9, 11) and immediate airway bronchoconstriction (12) in rodent models of allergic bronchopulmonary inflammation. Data from mice deficient in the third component of the complement system (C3) (13) and the complement anaphylatoxin receptor, C3aR (14), reveal diminished AHR, lung eosinophil levels, and reduced antigen-specific IgE and IgG1 responses. These results not only show a significant attenuation of the allergic inflammation in the absence of complement activation but also demonstrate a regulatory link between the C3a anaphylatoxin and Th2 effector functions.

In addition to these murine studies, which have focused predominantly on complement activation, C3a, and its receptor, another investigation demonstrated a significant genetic link between deficiency in the fifth component of the complement system (C5) and elevated AHR in experimental asthma (15). However, these studies were performed with noncongenic mouse strains so that the contribution of other genes to the AHR phenotype could not be ruled out. Moreover, other major biological features of asthma were not assessed in this report.

Therefore, to further elucidate the role of C5 in asthma, we have studied a murine model of allergic bronchopulmonary inflammation using congenic mice that are either sufficient (B10.D2/nSn) or deficient (B10.D2/oSn) in C5. In contrast to previous findings in C3- and C3aR-deficient animals, challenge of C5-deficient mice results in an exacerbated allergic response with elevated AHR, eosinophil recruitment, and Th2 cytokine production, and IgE responses compared with C5-sufficient animals. Collectively, these data demonstrate a novel role for C5 that is protective for allergic airway inflammation and suggest opposing regulatory functions for C3a and C5a in the development of Th2 responses in asthma. Some of the results presented here have been previously published in the form of an abstract (16).

METHODS

Mice

C5-deficient (B10.D2/oSn; C5$^{-/-}$) mice were obtained from Jackson Labs (Bar Harbor, ME). These mice have a 2-bp deletion in a 5' exon that causes C5 protein deficiency (17). The matching congenic C5-sufficient strain (B10.D2/nSn, C5$^{+/+}$; Jackson Labs) was used as the wild-type control for all described experiments. This work was conducted in accordance with institutional and National Institutes of Health guidelines.

Sensitization and Challenge Protocol

The mouse model used to induce allergic bronchopulmonary inflammation has been described (14). Four-week-old mice were exposed to a mixed antigen preparation consisting of *Aspergillus fumigatus* cell-culture filtrate prepared free of living organisms (18) and ovalbumin (OVA) (Sigma Chemical Co., St. Louis, MO). Addition of OVA to the preparation permitted detection of antigen-specific immunoglobulin levels after AHR measurements to ascertain immune responses during the protocol (19). Mice were sensitized intraperitoneally with the mixed

antigen preparation on Days 1, 5, 9, and 13 followed by two intranasal challenges on Days 17 and 19. AHR experiments were conducted on mice 24 h after their last antigen challenge. Animals were then exsanguinated, and blood was collected for total and OVA-specific immunoglobulin measurements. Bronchoalveolar lavage (BAL) fluid and lungs were also collected 24 h after the last challenge to assess cell differentials, levels of IL-5 and IL-13, numbers of IL-4– and IFN-γ–producing cells, and lung histology (14).

AHR Measurements

AHR to acetylcholine (ACh) provocation was measured as described (14, 20). Mice were anesthetized intraperitoneally with 20 μg/g etomidate (Abbott Laboratories, Abbott Park, IL). Trachea were cannulated, connected to a ventilator (Harvard Apparatus, Holliston, MA), and ventilated with 100% oxygen at a rate of 150 breaths/min and a tidal volume of 9 μl/g. After being paralyzed with 4 μg/g pancuronium bromide (Gensia Laboratories, Irvine, CA), mice were placed into a rodent plethysmograph capable of determining tidal volume, airflow, and transthoracic resistance (Buxco Electronics, Inc., Sharon, CT). AHR was monitored following intravenous tail injections of increasing log doses (μg/g) of ACh (Sigma). Airway responses were expressed as the concentration of ACh required to double baseline transthoracic resistance (PC_{200}).

C5 Inhibition Protocol

C5 activity was blocked in this model utilizing the previously described anti-mouse C5 monoclonal antibody (mAb) BB5.1 (21). Five-week-old C57BL/6J mice (Jackson Labs) utilized for these experiments were organized into four groups. Group 1 animals were sensitized and challenged with the mixed antigen preparation as described and were not treated with BB5.1. Group 2 animals had 40 mg/kg BB5.1 instilled intranasally 30 min before each challenge with antigen. Group 3 animals had 40 mg/kg BB5.1 injected intraperitoneally 30 min before each sensitization with antigen. Group 4 animals were treated with 40 mg/kg BB5.1 30 min before each sensitization and challenge with antigen. AHR was measured, and BAL fluid and lungs were collected 24 h after the last antigen challenge.

Statistical Analysis

Statistical analysis was performed using Prism software (Graphpad, San Diego, CA), and statistical significance was assessed using the two-tailed, unpaired Student's t test.

RESULTS

Effect of C5 Deficiency on AHR after Antigen Challenge

To address the role of C5 or its cleavage product, the C5a anaphylatoxin, on the function of Th2 cells during allergic airway inflammation, we subjected the congenic C5-deficient (B10.D2/oSn) and C5-sufficient (B10.D2/nSn) mice to an in vivo mouse model of pulmonary allergy. Twenty-four hours after the last antigen challenge, AHR to increasing doses of ACh was evaluated by airway plethysmography. Within this model, mice challenged with the antigen preparation are expected to have greater sensitivity to ACh, and, as a result, the concentration of ACh required to induce a 200% increase in airway resistance above baseline will be lower for these mice compared with control mice challenged with phosphate-buffered saline (PBS) (3). As shown in Figure 1, antigen-challenged wild-type mice developed an increase in AHR compared with the PBS control mice as revealed by the enhanced sensitivity when exposed to ACh. In antigen-challenged $C5^{-/-}$ mice, AHR sensitivity was not only increased relative to their PBS-challenged controls, it was also significantly greater than the ACh sensitivity observed for the challenged C5-sufficient mice. As such, the increased ACh sensitivity of antigen-challenged $C5^{-/-}$ mice demonstrates a greater level of AHR in the deficient mice compared with the antigen-challenged $C5^{+/+}$ animals.
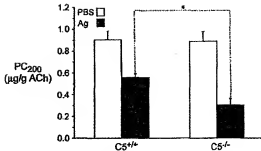

Figure 1. Effect of fifth complement component (C5) deficiency on airway hyperresponsiveness (AHR) in mice challenged with antigen. $C5^{+/+}$ and $C5^{-/-}$ mice were challenged via intranasal instillation on Days 17 and 19. Twenty-four hours after the last challenge, mice were anesthetized, intubated, and mechanically ventilated, and airway responses to increasing doses of intravenous acetylcholine (ACh) were assessed. AHR is expressed as the provocative concentration of ACh (in μg/g) that increased baseline airway resistance 200% (PC_{200}). Control mice, $C5^{+/+}$ or $C5^{-/-}$, were sensitized with the mixed antigen preparation and then challenged with phosphate-buffered saline (PBS). Results are plotted as means ± SEM (PBS-$C5^{+/+}$, n = 5; PBS-$C5^{-/-}$, n = 5; antigen-$C5^{+/+}$, n = 10; antigen-$C5^{-/-}$, n = 10), and significant differences between challenged sufficient and deficient mice are indicated as *p < 0.05 as determined by Student's t test. Ag = antigen.

Effect of C5 Deficiency on Inflammatory Cell Recruitment in Antigen-challenged Lungs

An additional facet of asthma is the pulmonary inflammation characterized by the presence of leukocytes, specifically eosinophils, recruited into the airways (22). Eosinophils are chemotactic to C5a (23), and mice deficient in C5 could potentially have reduced eosinophil recruitment to the lung after exposure to antigen. To determine if C5 contributes to eosinophil recruitment, lungs from challenged mice were lavaged after the AHR measurements, and the BAL fluid was examined for influx of leukocytes (Figure 2). Quantitation of leukocytes from the BAL fluid of PBS-challenged $C5^{+/+}$ and $C5^{-/-}$ mice revealed a population of cells comprised predominantly of macrophages with few neutrophils, eosinophils, and lymphocytes. Challenging wild-type mice with the mixed antigen preparation resulted in an increase in all cell types relative to PBS controls, and the BAL cell population consisted mainly of eosinophils and neutrophils. Challenging C5-deficient mice, however, revealed a significant elevation in the granulocyte population, including a significant 3.4-fold increase in eosinophils as well as a significant 2.0-fold increase in airway neutrophils. The increased presence of inflammatory cells in the BAL fluid was further corroborated by hematoxylin and eosin–stained lung sections from challenged $C5^{-/-}$ mice (Figure 3), which revealed increased infiltration of eosinophils and neutrophils into the peribronchial and parenchymal regions between the pulmonary blood vessels and the airways relative to their challenged wild-type counterparts (Figure 3). Collectively, these results demonstrate that mice deficient in C5 not only have increased lung eosinophil recruitment after antigen challenge but also have elevated neutrophil recruitment as well.

Effect of C5 Deficiency on Th2 Responses in Antigen-challenged Lungs

Previous reports have described elevated levels of Th2 cytokines in asthma (24) and the importance of the Th2 response in mouse models of pulmonary allergy (3, 4). To assess T-cell activation
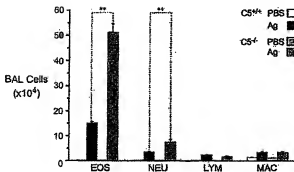
Figure 2. Effect of C5 deficiency on inflammatory cell recruitment in mice challenged with antigen. Quantitation of cells in the bronchoalveolar lavage (BAL) fluid from C5+/+ and C5−/− mice was assessed 24 h after the last challenge. Results are plotted as means ± SEM (PBS-C5+/+, n = 5; PBS-C5−/−, n = 5; antigen-C5+/+, n = 10; antigen-C5−/−, n = 10), and significant differences between sufficient and deficient mice are indicated as **p < 0.01 as determined by Student's t test. PBS controls shown were C5+/+ or C5−/− mice that were sensitized with the mixed antigen preparation and then challenged with PBS. Ag = antigen; EOS = eosinophils; LYM = lymphocytes; MAC = macrophages; NEU = neutrophils.

Figure 4. Effect of C5 deficiency on Th2 responses in mice challenged with antigen. (*A*) Numbers of interleukin (IL)–4–producing cells in the lungs from C5+/+ and C5−/− mice were quantitated by enzyme-linked immunospot assay 24 h after the last challenge. (*B*) BAL levels of IL-5 and IL-13 from C5+/+ and C5−/− mice were assessed by ELISA 24 h after the last challenge. Results are plotted as means ± SEM (PBS-C5+/+, n = 5; PBS-C5−/−, n = 5; antigen-C5+/+, n = 10; antigen-C5−/−, n = 10), and significant differences between sufficient and deficient mice are indicated as **p < 0.01 as determined by Student's t test. PBS controls shown were C5+/+ or C5−/− mice that were sensitized with the mixed antigen preparation and then challenged with PBS. Ag = antigen.

in C5−/− mice, IL-4 and IFN-γ production in the lung was quantitated by enzyme-linked immunospot assay (ELISPOT) and evaluated as a marker of Th2 versus Th1 responses after challenge (Figure 4A). Consistent with this model, wild-type mice challenged with the mixed antigen preparation exhibited a pronounced Th2 response in the lung as demonstrated by the elevated levels of cells expressing IL-4 and lack of cells expressing IFN-γ. Consistent with the elevated AHR and eosinophil recruitment observed in challenged C5-deficient mice, cells expressing IL-4 were also elevated 2.7-fold in the lungs of challenged C5−/− mice compared with the wild-type control. Although lungs from challenged wild-type mice had low levels of IFN-γ–producing cells (Figure 4A), lungs from either PBS-treated control animals or antigen-challenged C5-deficient mice did not have measurable IFN-γ–producing cells above the background limits for the assay, and analysis of BAL fluid by ELISA also revealed no detectable quantities of IFN-γ protein in these animals (data not shown).
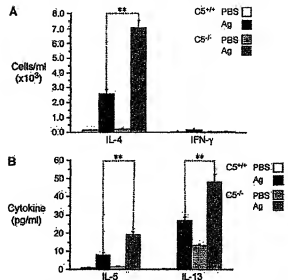
To further support the IL-4 ELISPOT observations, levels of IL-5 and IL-13 were measured because production of these cytokines is also indicative of a Th2 response (24). Analysis of these cytokines by ELISA revealed little or no IL-5 or IL-13 in the BAL fluid from PBS-challenged C5+/+ animals. Challenging C5+/+ mice with the mixed antigen preparation resulted in increased IL-5 and IL-13 levels (Figure 4B) relative to the PBS
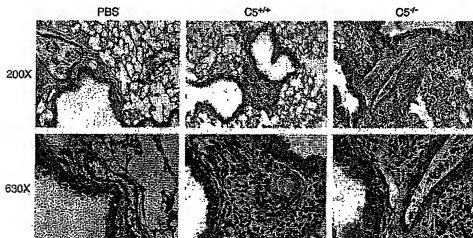

Figure 3. Effect of C5 deficiency on airway inflammation in mice challenged with antigen. Lungs from antigen-challenged C5+/+ and C5−/− mice were perfused with formalin and removed from the chest cavity 24 h after the last challenge. Tissues were embedded in paraffin, cut into 5-μm sections, stained with hematoxylin and eosin, and visualized by light microscopy. Sections shown are representative of three separate experiments. PBS controls shown were C5+/+ mice that were sensitized with the mixed antigen preparation and then challenged with PBS.

control animals. Challenging CS⁻/⁻ mice with the mixed antigen preparation elevated BAL IL-5 levels 2.4-fold and IL-13 levels 1.8-fold above challenged wild-type animals. These results and the increased numbers of IL-4–producing cells demonstrate that C5 is involved in IL-4, IL-5, and IL-13 regulation in the lung and implicate a link between C5 and the adaptive immune response as evidenced by the heightened Th2 responses of the CS⁻/⁻ animals in this model.

Effect of C5 Deficiency on Antigen-specific Immunoglobulin Levels

The preceding data suggest that the elevation in the airway responses and pulmonary inflammation observed in antigen-challenged CS⁻/⁻ mice is due to a defect in Th2 function. To gain further insight into the mechanisms linking complement to adaptive immunity, we determined total and antigen-specific antibody responses in CS⁺/⁺ and CS⁻/⁻ mice. Increased production of IgE antibodies during the course of asthma is another characteristic of the disease (25), and examination of total serum IgE in antigen-challenged CS⁺/⁺ and CS⁻/⁻ mice revealed elevated levels of IgE compared with their PBS control mice. However, total IgE levels in challenged C5-deficient mice were significantly increased 2.1-fold compared with the similarly challenged wild-type mice (Figure 5). OVA-specific IgE, IgG1, and IgG2a isotypes were also examined in CS⁻/⁻ mice (Figure 6), and quantitation of OVA-specific IgE and IgG1 titers revealed an increase of 2.3-fold and 1.7-fold, respectively, compared with challenged wild-type control animals. These results are consistent with the premise that IgE and IgG1 production, which is controlled by Th2 production of IL-4 and IL-13 (26), should be increased as a result of elevated IL-4 and IL-13 production in the C5-deficient mice. Moreover, expression of IgG2a, which is regulated by Th1 cells and IFN-γ (27), would be decreased since very little or no IFN-γ was detected in challenged CS⁺/⁺ and CS⁻/⁻ mice. Collectively, these results strongly suggest that the increased IgE levels result from the elevated IL-4 and IL-13 production in the challenged C5-deficient mice and reveal a suppressive role for C5 in the regulation of IgE responses by Th2 cells.
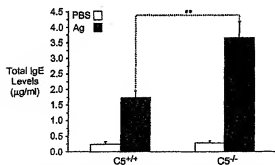
Effect of C5 Inhibition on AHR after Antigen Challenge

To determine if C5 activation exerted a suppressive role during sensitization or challenge, antigen sensitized and challenged mice were treated with the previously described anti-mouse C5 mAb BB5.1, which blocks C5 activity (21). As described in METHODS, mAb BB5.1 was administered to each mouse 30 min before each intranasal challenge with antigen, each intraperitoneal sensitization with antigen, and each sensitization and challenge. Twenty-four hours after the last antigen-challenge, AHR was measured. As shown in Figure 7, treatment with BB5.1 before challenge (group 2), sensitization (group 3), or both (group 4) significantly increased AHR relative to the wild-type control that was sensitized and challenged with antigen but not exposed to BB5.1 (group 1). Moreover, examination of lung histology revealed increases in airway inflammatory cell infiltrates relative to BB5.1 control mice (data not shown) that were verified by quantitation of total cells present in BAL fluid (BB5.1 control: 0.6 × 10⁴ ± 0.5 × 10⁴ cells/ml; group 1: 92.1 × 10⁴ ± 31.6 × 10⁴ cells/ml); group 2: 227 × 10⁴ ± 30.8 × 10⁴ cells/ml, p < 0.05; group 3: 274 × 10⁴ ± 39.9 × 10⁴ cells/ml, p < 0.05; group 4: 190 × 10⁴ ± 26.6 × 10⁴ cells/ml, p < 0.05).

DISCUSSION

In this report, we demonstrate that all features of the experimental asthma model, which include AHR, lung and airway recruitment of eosinophils, Th2 activation and recruitment to the lung, and IgE and IgG1 secretion by B cells, are elevated in the absence of C5. Blocking of C5 activity in wild-type mice utilizing an anti-C5 mAb during the sensitization or challenge phases of this model also caused increased AHR and pulmonary inflammation. These results indicate that C5 is a major modulator of allergic lung disease and establishes C5 as a regulator of Th2 development and effector functions. Together these data suggest that the complement C5a and its receptor (C5aR) provide a protective role during much of the allergic airway inflammation by tempering Th2 immune responses in asthma.

The data described here demonstrating the importance of C5 in regulating key biological features of asthma are supported by
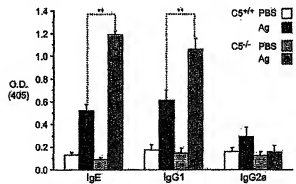
Figure 5. Effect of C5 deficiency on total IgE levels in mice challenged with antigen. Total serum IgE levels in CS⁺/⁺ and CS⁻/⁻ mice were assessed 24 h after the last challenge. Results are plotted as means ± SEM (PBS-CS⁺/⁺, n = 5; PBS-CS⁻/⁻, n = 5; antigen-CS⁺/⁺, n = 10; antigen-CS⁻/⁻, n = 10), and significant differences between sufficient and deficient mice are indicated as **p < 0.01 as determined by Student's t test. PBS controls shown were CS⁺/⁺ or CS⁻/⁻ mice that were sensitized with the mixed antigen preparation and then challenged with PBS. Ag = antigen.

Figure 6. Effect of C5 deficiency on antigen-specific IgE levels. OVA-specific IgE, IgG1, and IgG2a levels were quantitated from CS⁺/⁺ and CS⁻/⁻ littermates 24 h after the last challenge. Results are plotted as means ± SEM (PBS-CS⁺/⁺, n = 10; PBS-CS⁻/⁻, n = 5; antigen-CS⁺/⁺, n = 15; antigen-CS⁻/⁻, n = 15), and significant differences between wild-type and knockout mice are indicated as **p < 0.01 as determined by Student's t test. PBS controls shown were CS⁺/⁺ or CS⁻/⁻ mice that were sensitized with the mixed antigen preparation and then challenged with PBS. Ag = antigen; O.D. = optical density.
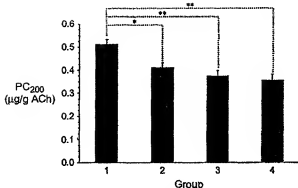
Figure 7. Effect of C5 inhibition utilizing the anti-mouse C5 monoclonal antibody (mAb) BB5.1 on AHR in mice challenged with antigen. Group 1 animals were sensitized and challenged and were not treated with the mAb. Group 2 animals were instilled intranasally with BB5.1 before each challenge with antigen. Group 3 animals were injected intraperitoneally with BB5.1 before each sensitization with antigen. Group 4 animals were treated with BB5.1 before each sensitization and challenge with antigen. Twenty-four hours after the last challenge, mice were anesthetized, intubated, and mechanically ventilated, and airway responses to increasing doses of intravenous ACh were assessed. AHR is expressed as the provocative concentration of ACh (in μg/g) that increased baseline airway resistance 200% (PC_{200}). Results are plotted as means ± SEM (group 1, n = 5; group 2, n = 5; group 3, n = 5; group 4, n = 5), and significant differences between untreated and BB5.1-treated mice are indicated as *p < 0.05 and **p < 0.01 as determined by Student's *t* test. Concentrations of ACh required to double baseline airway resistance in PBS (1.02 ± 0.07 μg/g ACh) and BB5.1 alone (0.99 ± 0.02 μg/g ACh) control mice were similar to values observed in the PBS control mice for $C5^{+/+}$ and $C5^{-/-}$ comparisons shown in Figure 1.

previous studies examining the role of C5 in human asthma patients. Many reports have documented the presence of C5a, an anaphylactic peptide originating from proteolytic cleavage of C5 during complement activation, in the lungs of asthmatics after provocation with allergen (8, 28, 29). Furthermore, a haplotype of the C5 gene has been identified as a susceptibility locus in asthma and described as protective against childhood and adult bronchial asthma (30). In addition to these human studies, rodent models of allergic airway disease also support our findings. A 2-bp deletion in the coding sequence of the mouse C5 gene has been linked to AHR susceptibility (15) and the C5aR gene has been localized to a region on mouse chromosome 7 that contains the allergen-induced AHR locus Abhr2 (31).

Collectively, these studies predict that C5 and its C5a activation fragment have a protective impact on the development of key biological hallmarks of asthma. In contrast, two recent publications, which utilized C5aR inhibitors in rodent models of experimental asthma, have indicated that C5a promotes allergic airway inflammation in the late or effector phase of the allergic response (32, 33). In the first publication, a hexapeptide C5aR antagonist (NMePhe-Lys-Pro-dCha-Trp-dArg) reduced the late airway response, eosinophil and neutrophil infiltration, and the induction of cytokine mRNA in the lungs of rats challenged with OVA (32). In the second report, an anti-C5aR mAb impaired AHR, eosinophil, neutrophil, and lymphocyte infiltration, and IL-4 production in the lungs of BALB/c female mice challenged with *A. fumigatus* antigen (33). These differences could be explained by inherent limitations in the controls employed for the inhibitor studies. For example, the interaction and inhibi-

tion of other receptors in the mouse by the hexapeptide was not examined. In addition, the interaction of the mAb with Fc receptors in the mouse model of experimental asthma was not assessed. The ability of the anti-C5aR mAb to bind FcγRIIB1 would merit particular attention because it acts as a potent suppressor of pulmonary inflammation (34, 35).

Possibly a more compelling explanation for the differences observed between the C5-deficient studies and those using C5aR inhibitors is the impact of C5 on the sensitization phase of the experimental allergic lung disease mouse model. Our studies and those of others using C5-deficient mice lack C5a during the sensitization and challenge phase, whereas the studies utilizing the C5aR inhibitors only blocked C5a during the challenge phase (32, 33). However, the mAb anti-C5 blocking data presented here indicate that C5, and possibly C5a, are important in both the sensitization and challenge phases of experimental lung disease, with inhibition of C5 activation at the time of sensitization or challenge resulting in elevated AHR and airway inflammation. Collectively, the mAb and C5 deficiency studies strongly suggest that chronic administration of inhibitors blocking C5 activation before and during the onset of an asthma attack would have the potential to exacerbate the disease. Therefore, caution should be used when evaluating C5 and C5aR inhibitors in the treatment of human asthma.

In conclusion, the study presented here substantiates a previous report describing C5-mediated regulation of AHR and documents a novel regulatory role for C5 in the development of Th2 responses in a mouse model of allergic airway disease. These results lay the foundation for additional studies directed at assessing the role of C5a and C5aR on CD4+ T lymphocytes *in vitro* as well as delineating *in vivo* the mechanisms by which C5a contributes to airway responses, lung eosinophil recruitment, and IgE production. Given the important contribution of Th2 cytokine production in asthma, further study of C5a-mediated regulation of T lymphocytes may shed light in the causative events leading to AHR and airway inflammation in this disease.

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